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A WNT/β-CATENIN NEGATIVE FEEDBACK LOOP INHIBITS IL-1β INDUCED MMP EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Recent animal studies suggest that activation of Wnt/β-catenin signaling in articular chondrocytes might be a driving factor in the pathogenesis of osteoarthritis (OA) by stimulating amongst others the expression of matrix metalloproteinases (MMPs). Indeed in animal chondrocytes IL-1β induced MMP expression is mediated by activation of canonical Wnt/β-catenin signaling. This study aimed to investigate the role of Wnt/β-catenin signaling in IL-1β induced MMP expression in human chondrocytes.
Methods: Primary cultures of human articular chondrocytes derived from post mortem healthy donors or patients with end stage osteoarthritis or rheumatoid arthritis were used in all experiments. Cells were stimulated with recombinant growth factors and cytokines. Changes in gene and protein expression were studied using qPCR, Western blot, and enzymatic essays. Multiple strategies for activation and inhibition of signaling pathways in human chondrocytes, such as lentiviral mediated gene overexpression or gene knock down were used. Luciferase reporter assays and co-immunoprecipitation essays were used to study the interaction between β-catenin and NF-κB.
Results: IL-1β potently stimulated MMP1, -3 and -13 expression in human and murine chondrocytes dose-dependently. In marked contrast, co-stimulation with the recombinant Wnt3A, which activates canonical Wnt/β-catenin signaling, potently inhibited IL-1β induced MMP dose-dependently in human chondrocytes but not in murine chondrocytes. This inhibitory effect was found in human chondrocytes irrespective of the disease state of the donor being either healthy, osteoarthritic or rheumatoid arthritic and was also found in human bone marrow derived mesenchymal stem cells. In contrast, Wnt/β-catenin signaling induced MMP expression in chondrocytes of all animal species tested, revealing an unprecedented species difference. Wnt3A's inhibitory effect was found in both basal conditions and after IL-1β stimulation at the mRNA level, protein level and in enzymatic essays. Wesetern blot indicated that IL-1β indirectly activated β-catenin in human chondrocytes. This was due to IL-1β induced upregulation of the canonical Wnt7B and down regulation of the Wnt-signaling antagonists DKK1, Wif1 and FRZb as demonstrated by gene knock down and gene overexpression studies. The inhibitory effect of Wnt3A on IL-1β induced MMP expression was independent of TCF/LEF transcription factors, since knock down of TCF4 did not alter Wnt3A's effect. This was in marked contrast to murine chondrocytes in which knock down of TCF4 completely blocked IL-1β induced MMP expression. Subsequently, we showed that the inhibitory effect on IL-1β induced MMP expression in human chondrocytes was due to an inhibitory protein-protein interaction between β-catenin and NF-κB/p65RELA blocking the activation of NF-κB promoter reporter constructs as well as inhibiting the expression of established NF-κB target genes like IL-6.
Conclusions: Our study reveals an unexpected and unprecedented species difference between human and animal chondrocytes with respect of the role of Wnt/β-catenin signaling in the regulation of MMP expression. In marked contrast to mouse chondrocytes in which Wnt/β-catenin signaling is part of an IL-1β activated pro-catabolic sequence resulting in increased MMP expression, in human cells β-catenin has an unexpected anti-catabolic role. We provide evidence that Wnt/β-catenin signaling is part of an IL-1β activated negative feedback loop involving upregulation of the canonical Wnt7B and down regulation of established Wnt-antagonists like DKK1. This leads to activation of β-catenin, which in turn inhibits NF-κB activity due to an inhibitory protein-protein interaction with p65RELA. Our results question the relevance of animal models for studying the role of Wnt/β-catenin signaling in osteoarthritis. They furthermore imply that upregulated β-catenin in human osteoarthritic cartilage may be part of an anti-catabolic response counteracting pro-catabolic NF-κB signaling.

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BMP-9 INDUCES BOTH SMAD1/5/8 AND SMAD2/3 PHOSPHORYLATION, AND SPECIFIC RESPONSE GENES, IN CHONDROCYTES

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Background: Osteoarthritis is characterized by loss of articular cartilage. The main cartilage degrading enzyme is Matrix metalloproteinase 13 (MMP13). Expression of MMP13 is positively correlated with Activin receptor-like kinase 1 (ALK1) expression. Moreover, terminal differentiation of chondrocytes is induced by ALK1 via phosphorylation of Smad1/5/8 (Smad1/5/8p). BMP-9, also known as GDF-2, has recently been characterized as a potent, high-affinity ALK1 ligand, that is present in serum in high amounts . We wanted to further investigate intracellular BMP-9 signaling in chondrocytes to clarify its role in cartilage maintenance and degradation.
Purpose: To study Smad1/5/8 and Smad2/3 phosphorylation kinetics in response to addition of exogenous BMP-9, to investigate BMP-9's potency on chondrocytes, and to investigate downstream signaling responses on mRNA level.
Methods: Both primary bovine chondrocytes, isolated from the metacarpophalangeal joint of 2 year old animals, as well as the murine H4 chondrocyte cell line were used in this study. Chondrocytes were cultured to near confluency, and subsequently incubated with BMP-9. After short term stimulation (1 h), Smad phosphorylation was analyzed by specific Smad2/3p and Smad1/5/8p staining of Western blots. After long term stimulation (24-48 h), expression of PAI-1, a downstream marker of Smad2/3 signaling, and ID-1, a downstream marker of Smad1/5/8 signaling, were analyzed by quantitative real time PCR (qPCR).
Results: In both the murine cell line, as well as the primary bovine chondrocytes, addition of BMP-9 resulted in phosphorylation of Smad 1/5/8, that was maximal at very low concentrations (100 pg / ml). Unexpectedly, Smad2/3p was also observed at this concentration. Further investigations showed that BMP-9-induced Smad2/3p was maximal after 1 h, at the physiological relevant concentration of 5 ng/ml. Exposure of chondrocytes to BMP9 not only resulted in both Smad1/5/8p and Smad2/3p but also in transcription of pathway specific response genes, as observed by a significant increase in PAI-1 and ID-1 mRNA levels after both 24 and 48h. In both cell types, collagen type 2A1 and aggrecan mRNA levels were also up regulated by BMP9. In the described experiments, no significant changes in MMP13 expression were observed after 48 h of BMP-9 stimulation.
Conclusion: In chondrocytes, BMP9 is capable of inducing both Smad1/5/8p and Smad2/3p. By doing so it can up regulate both PAI-1 and ID-1 simultaneously. BMP-9 is very potent in inducing Smad phosphorylation, as very low concentrations (pg/ml) are sufficient for signal induction. The induction of Smad2/3p and subsequent PAI-1 activity sets BMP-9 apart from other BMPs, like BMP-2 and BMP-6, that only induce Smad1/5/8 phosphorylation. The lack of MMP13 expression after BMP-9 stimulation can be explained by the induced Smad2/3p expression, as this potently blocks MMP13 transcription. Based on these results we propose that exposure of young cartilage to BMP-9 does not result in MMP13 expression due to its ability to induce Smad2/3p. However, we previously reported that in ageing cartilage the ability to phosphorylate Smad2/3 is reduced, and therefore BMP-9 exposure will most likely result in MMP13 production in old cartilage.

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A TYPE II COLLAGEN PEPTIDE ACTIVATES P38 MITOGEN-ACTIVATED PROTEIN KINASE AND ITS INHIBITION BY HYALURONAN VIA ICAM-1 IN ARTICULAR CHONDROCYTES

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Background: Some proteolytic products of cartilage matrix may contribute to cartilage destruction through their catabolic activities. Recently, we have found that a 24-mer synthetic peptide of type II collagen named CB12-II stimulates type II collagen cleavage with induction of